Document No.: 9530-035-04

Biodegradation Feasibility Analysis of Wastewater Lagoons UOP Site, East Rutherford, New Jersey

Prepared for:

UOP, Inc.
Des Plaines, Illinois

March 1988

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March 21, 1988

Christine Altomari
Case Manager
N.J. Department of Environmental Protection
Division of Hazardous Waste Management
401 E. State St., 5th Floor West
CN 028
Trenton, NJ 08625

Dear Ms. Altomari:

I am enclosing 7 copies of the ERT Report titled "Biodegradation Feasibility Analysis of Wastewater Lagoons" for the UOP, Inc. Superfund site in East Rutherford, New Jersey. This report is submitted in fulfillment of implementation of the biodegradation feasibility analysis which began in August 1987. It is part of the work performed under the requirements of an Administrative Consent Order (ACO) signed in May 1986 by UOP, Inc. and the NJDEP.

Please note that only 1 set of the data contained in Appendices A through D is included in the large 4-inch volume, which also contains the report. Six copies of the report without the data appendices are also included.

If there are any questions, please do not hesitate to call Mr. Lawrence Geyer at (312) 391-2675.

Sincerely,

Richard Woodward /l.s.

Richard Woodward, Ph.D
Senior Project Scientist

RW:dg:9530-035

cc: Lawrence A. Geyer, P.E., UOP, Inc.
Michael Worthy, ERT, Inc.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF APPENDICES

LIST OF ILLUSTRATIONS

TABLE OF CONTENTS (Continued)

LIST OF ILLUSTRATIONS (Continued)

LIST OF TABLES

TABLE OF CONTENTS (Continued)

LIST OF TABLES (Continued)

TABLE OF CONTENTS (Continued)

LIST OF TABLES (Continued)

EXECUTIVE SUMMARY

A biodegradation feasibility analysis was conducted to evaluate the biodegradability of organic constituents in the sludges and meadow mat (a high organic matter peat-like layer of plant origin) layers of two wastewater lagoons at the UOP site in East Rutherford, NJ. The physical, chemical, and biological characteristics of the two layers were defined in order to select appropriate treatment conditions and to identify appropriate analyses for monitoring the progress and activity of biodegradation.

Samples of sludge alone, and naturally occurring proportions of sludge and meadow mat, were treated with six nutrient regimes. The treatments were evaluated according to changes in toxicity, chemical oxygen demand, and microbial enzyme activity. Biodegradation was confirmed by quantitative analyses of hydrocarbon oil and grease and benzene, toluene, and xylene. The highest nutrient concentration, (a 5:1, nitrogen to phosphorus ratio), provided for the greatest stimulation of biological activity. This treatment stimulated biodegradation in sludge alone and in the sludge/meadow mat combination. This treatment was used in a scaled-up test designed to evaluate biodegradation in the matrix likely to be used in the field.

A mixture similar to the naturally occurring ratio of sludge and meadow mat from the most highly contaminated area of lagoon 1 was chosen for the scaled-up test. The mixture was treated with nutrients, and then divided into two portions. One was incubated at 100% field moisture capacity to simulate in situ biodegradation in lagoon 1. A second was allowed to dry naturally to 50 to 70% field moisture capacity to simulate traditional land treatment conditions. HSL plus 30 analyses before and after 35 days of incubation confirmed highly significant reduction in HSL organics, in hydrocarbon oil and grease, and in overall toxicity.

Changes in the concentration of the tentatively identified compounds (extra peaks) were consistent with changes in the concentration of the HSL compounds. This indicated that compounds resistant to biodegradation did not accumulate and that HSL constituents were not simply "delisted" by biological transformation to other organics. Both wet and dry treatments provided for rapid biodegradation of the organic constituents. Dry treatment provided for the highest biodegradation rate and the lowest residuals after 35 days. Based on these results, recommendations were made for on-site bioremediation using conditions that would limit the moisture content to 70% field capacity.

1.0 INTRODUCTION

The UOP Superfund site is located in East Rutherford, NJ. Investigations, studies, and remedial actions concerning the site are performed under the requirements of an Administrative Consent Order (ACO) signed in May 1986 by UOP, Inc. and the New Jersey Department of Environmental Protection (NJDEP). Because of the varying physical characteristics and progress of prior activities in different parts of the property, the site was divided into six areas identified in the May 1986 ACO. This report pertains to one of those areas: Area 3, which consists of two former wastewater lagoons. The two lagoons are located side by side on the site and their combined surface area (including berms) is approximately 1.2 acres.

The May 1986 ACO required that a decision be made regarding the lagoons: to either proceed with plans to excavate and dispose of the lagoons' contents in an off-site landfill or to perform a feasibility study to evaluate possible remedial alternatives. Initially, the choice was made to proceed with the excavation and off-site disposal program, which progressed to publication of the document entitled: "Wastewater Lagoons, Remedial Action Work Plan, Revision 1," February 1987. Shortly after submittal of this Work Plan, two factors compelled consideration of a different approach toward lagoon remediation:

- the Superfund Amendments and Reauthorization Act (SARA) required greater use of permanent technologies and less use of transportation and off-site disposal of wastes from Superfund sites; and

- UOP discussions with several industry experts indicated that bioremediation for the lagoons' materials appeared to be feasible.

With these factors in mind, UOP approached the NJDEP concerning the possibility of considering the feasibility of bioremediation, while postponing further consideration of the excavation and off-site disposal plan. The NJDEP consented to the new approach. Consequently, ERT, Inc. prepared a Work Plan entitled: "Wastewater Lagoons Biodegradation Feasibility Analysis and Design Optimization Work Plan," which was submitted to the NJDEP in July 1987. Implementation of the feasibility analysis began in August 1987 and ends with the submission of this document.

This document presents the results of the biodegradation feasibility analysis of sludge and meadow mat samples collected from the two wastewater lagoons (1 and 2). It also describes the entire feasibility analysis. This ranges from sample collection, characterization, primary biodegradation screen, and scaled-up test through data reduction and interpretation. Summary recommendations for the field implementation are also presented.

The overall objective of this study was to evaluate the feasibility of biodegradation as an approach to remediation. The study focused on the organic constituents of the sludge and meadow mat layers of the wastewater lagoons 1 and 2 at the UOP site. The specific study objectives were:

1. to define the loading capacity for sludges from each lagoon;

2. to identify factors limiting the activity of the indigenous microflora;

3. to evaluate the effect of the meadow mat layer on biodegradation;

4. to identify some appropriate measurements of biodegradation activity and progress;

5. to compare the rate and extent of biodegradation under wet and dry conditions; and

6. to develop recommendations for field implementation.

2.0 SAMPLE COLLECTION

2.1 Background Information

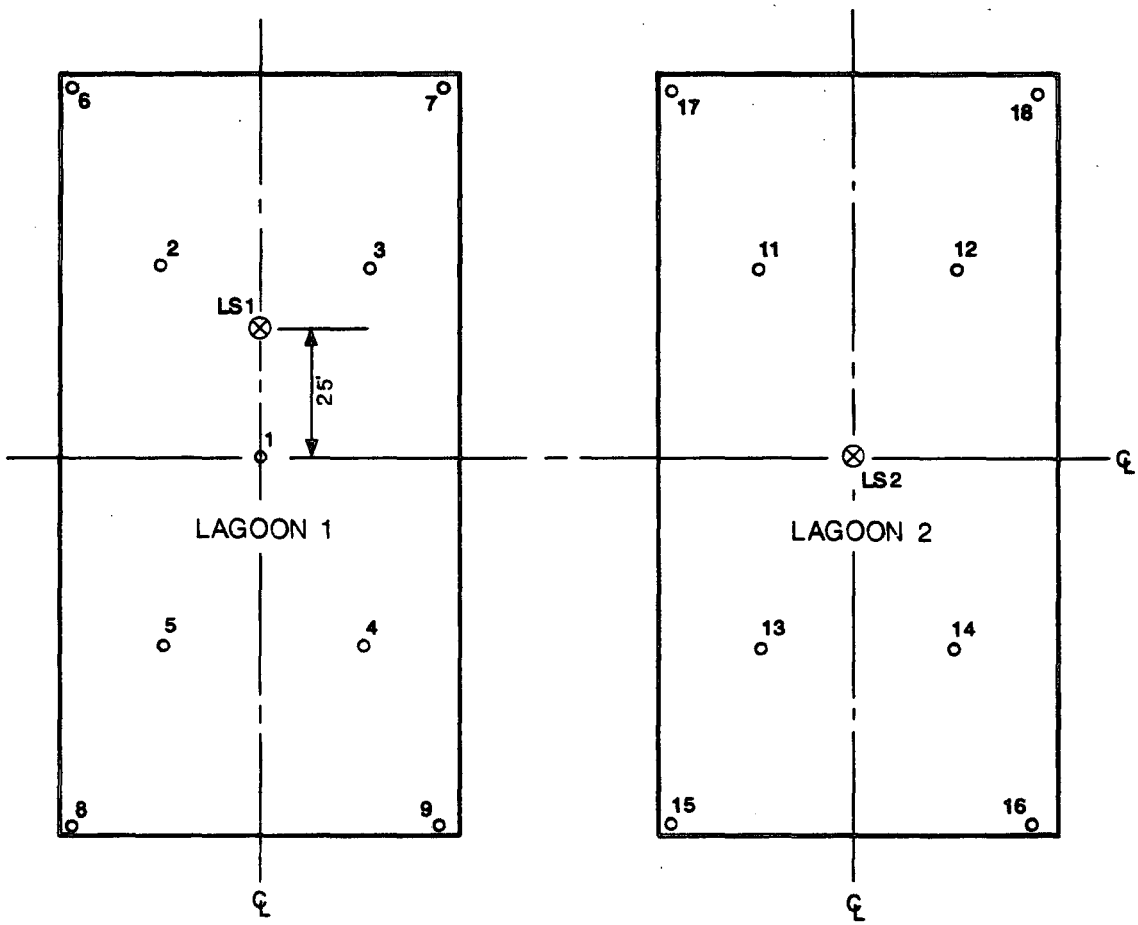
The objective of sample collection was to obtain enough sludge and meadow mat from Lagoon 1 and Lagoon 2 to perform the three basic components of the feasibility analysis: Sample Characterization, Primary Screen, and Scaled-Up Test. An additional objective was to collect a soil sample from a clean area on site to use as loading material. This sample was collected, but not used in the feasibility analysis.

The sampling effort took place on August 3 and 4, 1987. Preparations began on August 3, while the actual collection of samples from Lagoons 1 and 2 took place on August 4. The following sections describe the sample locations, sample procedures, and significant field observations made during sample collection.

The required sampling, decontamination, and chain-of-custody procedures are contained in the Feasibility Analysis Work Plan (Ref. P-E197-311A).

2.2 Sample Locations

The sample locations are shown in Figure 2-1. These locations were selected with the goal of obtaining samples with the highest expected contaminant concentrations in both the meadow mat and sludge from one location in each lagoon. These locations were determined by examining the contaminant concentration distributions reported in the Phase II Investigation Report. The center point of Lagoon 2 was the selected location for Sample LS2. For Lagoon 1, the selected sample location was 25 feet north (along the long axis of the lagoon) of the center point. The center point of each lagoon was



LEGEND

- ○ PHASE II SAMPLING LOCATION
- ⊗ SAMPLING LOCATION FOR FEASIBILITY ANALYSIS

0 40 80

SCALE IN FEET

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FIGURE 2-1

LAGOON SAMPLING LOCATIONS
EXISTING PHASE II LOCATIONS &
LOCATIONS FOR FEASIBILITY STUDY SAMPLES
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determined by measuring the length of each dike along the perimeter of the lagoons, then measuring the mid-point of each dike, and finally finding the intersection of the lines between opposing mid-points.

The measurement of each dike length proceeded as described above except for the dike on the east side of Lagoon 2. The southern end of this dike could not be located because of vaguely defined topography and thick vegetation. As an alternative measure, the mid-point on the eastside dike was assumed to be the same distance from the north dike as was measured on the west-side dike.

Each sample location was marked with a 6 foot-long metal stake placed at a 5-foot offset south of the sample location.

2.3 Sample Technique

The following procedure was used to collect the sludge and meadow mat samples:

1. Phragmites roots were removed with a shovel to a depth of six inches.

2. There were 3.5 gallons of sludge collected from each lagoon using a post-hole digger; both the sludge and meadow mat samples were placed in plastic buckets with sealed lids.

3. Approximately 2 gallons of meadow mat were collected from Lagoon 1 using a standard bucket auger. It was intended that 3.5 gallons be collected, but the meadow mat layer was too thin (0.3 ft) to make this feasible.

4. There were 3.5 gallons of meadow mat collected from Lagoon 2 using a stainless steel core barrel.

5. A 3.5-gallon diluant sample of soil, to be used for providing loading capacity, was collected from the extreme southwest portion of the site. However, this sample was not used in the feasibility analysis.

6. All samples were stored on ice in coolers and shipped by overnight service to the ERT Houston office.

2.4 Field Observations

The following observations were made during the sampling event:

- HNu Readings
  - Sample Location LS1
    In six-inch excavation = 0
    In two-foot excavation = 1 ppm

- Material Thickness Encountered at Sample Locations
  - LS1, Lagoon 1, Sludge = 5.6 ft,
    Meadow Mat = 0.3 ft.
  - LS2, Lagoon 2, Sludge = 3.3 ft,
    Meadow Mat = 1.7 ft.

- Weather Conditions
  - August 3, mid-day air temperature in lagoons area = 85-90°F, high humidity, mostly sunny
  - August 4, mid-day air temperature in lagoons area = 85-90°F, slightly less high humidity, mostly sunny a.m., mostly cloudy p.m.

- Sample Times (all on August 4)
  - Lagoon 1, Sludge = 1:30-2:00 p.m.
    Meadow Mat = 2:00 p.m.-4:00 p.m.
  - Lagoon 2, Sample = 10:15-11:15 a.m.
    Meadow Mat = 11:15 a.m.-1:30 p.m.
  - Diluant Sample = 4:00-5:00 p.m.

- Tide Conditions
  - Ackerman's Creek was empty at 8:00 a.m., August 4, except for a very small ebb-flowing channel.

NOTE: Sampling in Lagoon 2 was performed in the morning to take advantage of the dry conditions due to low tide.

3.0 SLUDGE AND MEADOW MAT CHARACTERIZATION

3.1 Objective

A thorough physical, chemical, and biological characterization was made of the sludge and meadow mat samples from each lagoon. The objective of the characterization phase was to develop a comprehensive data base that would provide guidance:

1. to address potentially limiting factors, such as nutrient deficiencies or pH that would prevent or delay the progress of biodegradation;

2. to manage inhibitory factors such as metabolic poisons or metals that could interfere with timely biodegradation;

3. to select the most appropriate treatment matrix, either liquid or solid, for subsequent experimental designs;

4. to identify the best general analyses for monitoring activity and progress of biodegradation; and

5. to define the initial loading capacity of each sludge and meadow mat.

The analyses were performed on one sludge and one meadow mat sample from each of the two lagoons, for a total of four samples per analysis. A summary of the analytical methods used is shown in Table 3-1. Copies of the original laboratory data with appropriate quality assurance/quality control are presented in Appendix A.

TABLE 3-1

PHYSICAL AND CHEMICAL ANALYSES
FOR SAMPLE CHARACTERIZATION

Analysis	Analytical Method*
Physical Parameters	
Total Solids	Standard Method 109R
Fixed and Volatile Solids	Standard Method 209D
Chemical Parameters	
pH	Standard Method 423
Total Kjeldahl Nitrogen	Standard Method 420
Nitrate Nitrogen	Standard Method 418
Ammonical Nitrogen	Standard Method 417
Phosphorous, Total	Standard Method 424
Petroleum Oil & Grease	Standard Method 503A
Benzene, Toluene, Xylenes (BTX)	EPA Method 8020
Chemical Oxygen Demand (COD)	Standard Method 508A
Chlorides	Standard Method 407A or 407B
Specific Conductance	Standard Method 205
Copper	Standard Method 303R
Biological Parameters	
Microtox EC_{50}	See Appendix D
Standard Microbial Plate Count	EPA Method 3-A

*References: Standard Method = Standard Methods for the Examination of Water and Wastewater, 16th Ed., 1985

EPA Method = For BTX: EP-600/4-79-20; For Microbial Plate Count: EPR-600/8-78-017

3.2 Physical Characterization: Results and Discussion

Data from the physical analyses of sludge and meadow mat from Lagoon 1 and Lagoon 2 are summarized in Table 3-2. Sludges from both lagoons contained a higher percentage water and a lower percentage solids than their corresponding meadow mats. About 75 percent of the solids in Sludges 1 and 2 and Meadow Mat 2 were fixed solids (stable to 550°C). Meadow Mat 1 contained 80% fixed solids. These data suggest that biodegradation in a solid matrix would be preferred. Liquid matrix systems are ineffective at suspending a matrix with >10% solids. Removal of the volatile solids, usually interpreted to be organics, results in a weight reduction of less than 7 percent.

3.3 Chemical Characterization: Results and Discussion

Table 3-3 summarizes the inorganic and organic chemical analyses of the sludge and meadow mat samples. The pH of both sludges and meadow mats is within the acceptable range for biodegradation. In many similar systems, the pH becomes more acidic as biodegradation progresses.

The concentrations of chloride and copper, both potential inhibitors of biodegradation, are well below the levels usually associated with microbial inhibition (generally >5000 ppm). Furthermore, the solubility of copper in this alkaline environment would be very low. Both the sludge and meadow mat matrices in Lagoon 2 exhibit five times the chloride concentration of their counterparts in Lagoon 1. This is consistent with the observed breach in the Lagoon 2 dike and its hydraulic connection with seawater typically containing 18,980 ppm chloride. In both lagoons, the concentration of chloride in

TABLE 3-2

PHYSICAL CHARACTERIZATION OF SLUDGE (S1, S2) AND MEADOW MAT (M1, M2) COLLECTED FROM LAGOON 1 AND LAGOON 2

	Analytical Summary				
	Sludge		Meadow Mat		$\bar{x}$
Sample Matrix/Lagoon:	S1	S2	M1	M2	
% Water (w/w)*	78	73	69	69	72
% Solids (w/w)	22	27	31	31	28
% Volatile Solids (w/w)	25	24	19	25	23
% Fixed Solids (w/w)	75	76	81	75	77

*(w/w) indicates weight/weight relationship

TABLE 3-3

CHEMICAL CHARACTERIZATION OF SLUDGE (S1, S2)
AND MEADOW MAT (M1, M2) COLLECTED FROM
LAGOON 1 AND LAGOON 2

	Analytical Summary			
	Sludge		Meadow Mat	
Analysis Matrix/Lagoon:	S1	S2	M1	M2
Inorganic Parameters				
pH	8.50	8.95	7.50	7.95
Specific Conductance (umhos/cm)	2500	1800	220	1400
Cl (mg/kg)	382	2283	687	3676
Cu (mg/kg)	177	258	92	287
NO_3 (mg/kg)	103	77.8	246	220
NH_3 (mg/kg)	29.1	61.2	369	642
TKN (mg/kg)	886	1320	7711	8043
P (mg/kg)	106	2320	110	604
Organic Parameters				
Hydro O&G (mg/kg)	5327	3667	1825	2344
Benzene (ug/kg)	14000	460	8750	6100
Toluene (ug/kg)	22000	440	84000	2500
Xylenes (ug/kg)	4700	1200	5700	14600

the meadow mat is 1.6 to 1.8 times higher than in the corresponding sludge. The apparent chloride accumulation may be associated with the metabolic activity of the marsh grass associated with the meadowlands. Many plants growing in salty to brackish environments actively eliminate selective ions like chloride.

The specific conductance of sludge and meadow mat samples from Lagoon 1 was higher than those from Lagoon 2. However, the concentration of chloride was 5 to 6 times higher than the corresponding fraction from Lagoon 1. This apparent discrepancy was attributed to two possibilities. Ions other than those analyzed could be responsible for the difference in specific conductance. An ion balance analysis would reveal the contribution of other ions to the specific conductance value. A second possibility is related to the determination of specific conductance on a solid. Considerable variability exists in this analysis because of its dependence on the complete extraction and equilibration of ions from the sample. Incomplete desorption and ion interaction yields low specific conductance values.

The concentration of the essential nutrients nitrogen and phosphorous in both sludges and meadow mats is sufficient to support microbial growth. However, only a small portion of the total nitrogen (less than 13%) is available as nitrate or ammonia. Most nitrogen is bound as organic nitrogen in the meadow mat fraction of each lagoon. Sludge 2 contains an unusually high level of phosphorus. Typical phosphorus concentrations in soil range to several hundred mg/kg.

Results of the organic chemical characterization are consistent with earlier findings (Geraghty and Miller, 1985 Phase II Investigations). Lagoon 1 contained the highest concentration of BTX in both sludge and meadow mat fractions. Significant levels of hydrocarbon oil and grease were found in sludges and

meadow mat samples from both lagoons. These results indicate that both hydrocarbon oil and grease and BTX would be useful indicator analyses to track biodegradation progress.

3.4 Biological Characterization: Results and Discussion

Data for the biological characterization of both sludges and meadow mats are summarized in Table 3-4. Microorganisms were enumerated according to standard dilution plating methods on nutrient agar. The meadow mat contained two orders of magnitude or 100 times more colony-forming units/g fresh weight than the sludge.

The relative toxicity of the sludge and meadow mat samples was characterized with the Microtox bioassay. A complete description of the Microtox assay procedure is provided in Appendix D. Three stock dilutions (3.0%, 6.25% and 12.5% w/w) of each sludge and each meadow mat sample were made. This was done by mixing the sample with an appropriate amount of water in a blender (3 cycles of 1 min. on, 1 min. off). The relative toxicity of four serial dilutions of each stock dilution was measured with the Microtox instrument. The respective gamma values were plotted and the EC_{50} value determined graphically. The EC_{50} values for the three stock dilutions of each sample are shown in Table 3-4 and Figure 3-1. Sludge 1 was the most toxic fraction tested. It did not exhibit a reduction in toxicity within the dilution range analyzed. The toxicity of the meadow mat fractions from both lagoons decreased with increasing dilution. Sludge 1, however, was more toxic than its corresponding meadow mat. Sludge 2 was less toxic. Recall that the Effective Concentration (EC_{50}) is an inverse relationship with the toxicity of the test material. Highly toxic compounds have a very low Effective Concentration or EC_{50}.

TABLE 3-4

BIOLOGICAL CHARACTERIZATION OF SLUDGE (S1, S2)
AND MEADOW MAT (M1, M2) COLLECTED FROM
LAGOON 1 AND LAGOON 2

Analysis	Analytical Summary			
	Sludge		Meadow Mat	
Matrix/Lagoon:	S1	S2	M1	M2
Plate Count CFU x 10^6/ml	1.4	2.3	190	99
Microtox Bioassay EC_{50}				
Stock Solution				
3.00%	2.5	28.0	19.0	14.0
6.25%	2.5	25.0	8.0	11.0
12.50%	2.5	23.0	6.0	10.0

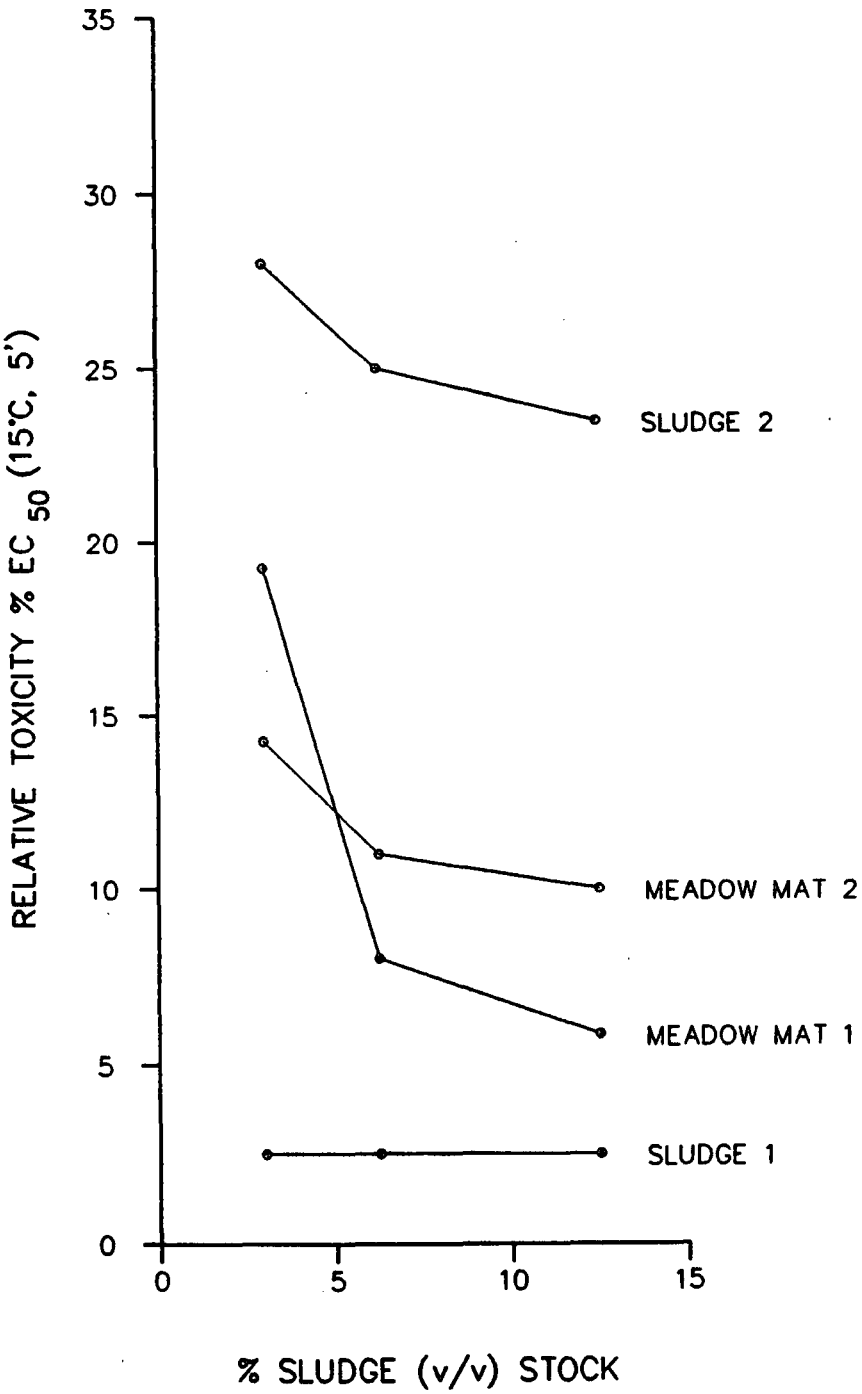

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FIGURE 3-1

BIOLOGICAL CHARACTERIZATION OF SLUDGE AND MEADOW MAT FOR TOXICITY

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4.0 PRIMARY BIODEGRADATION SCREEN

The objective of the primary biodegradation screen is to identify the specific nutrients, nutrient ratios and concentrations that stimulate indigenous organisms to a rapid biodegradation rate. A second objective is to evaluate some general and specific analyses for monitoring the activity and progress of biodegradation.

Sludge 1 was selected for the primary biodegradation screen. It had the highest concentration of hydrocarbon oil and grease, and exhibited the highest overall toxicity or worst case condition. The Sludge 2/Meadow Mat 2 combination was selected to evaluate the effect of meadow mat on the degradation process. It had less stringent toxicity conditions. Sludge 1 alone, and a mixture simulating the natural ratio of Sludge 2/Meadow Mat 2, was diluted to a concentration suitable for biodegradation. The effect of six nutrient regimes on biodegradation of each mixture was monitored by changes in toxicity, chemical oxygen demand, and catalase activity. The specific methods employed are discussed below.

4.1 Methods

A 9% (w/w) suspension of Sludge 1 in distilled water was homogenized by blending for 3 cycles of 1 min. on, 1 min. off. Likewise, a second reaction mixture containing a 9% (w/w) suspension of Sludge 2 plus 2% (w/w) Meadow Mat 2 (4.5:1) was also prepared. Six subsamples of each reaction mixture were treated with a mixture of nitrogen and phosphate nutrients formulated from commercially available fertilizers. The nutrient ratios are shown in Table 4-1. The nutrient program consisted of

TABLE 4-1

TREATMENT MATRIX FOR THE PRIMARY SCREEN

Treatment	Nutrient Formulation Analysis ($N-P_2O_5-K_2O$)	Rate mg/l	Nutrient mg/l N	Nutrient mg/l P	N:P
Control	0 - 0 - 0	0	0	0	0
A	10 - 13.8 - 0	300	30	18	5:3
B	10 - 23 - 0	300	30	30	5:5
C	20 - 9.2 - 0	75	15	3	5:1
C_1	20 - 9.2 - 0	150	30	6	5:1
C_2	20 - 9.2 - 0	225	45	9	5:1

one untreated control, one concentration of a 5:3 (N:P) ratio, one concentration of a 1:1 ratio, and three concentrations of a 5:1 ratio. The pH of each treatment including the control was adjusted to pH 7.0. Filter paper-capped vessels were incubated at 22 to 24°C on a rotary shaker (45 rpm) for 14 days. At regular, predetermined intervals, samples were taken and analyzed for relative toxicity, using the Microtox bioassay, for COD, and for catalase activity, using the HMB system (Biotech International, Bellaire, TX). For additional discussion of the catalase assay, see section 4.2.3. Based on the results of these tests, the three most active treatments for each sludge or sludge/meadow mat mixture were selected for analysis of hydrocarbon oil and grease and BTX (benzene, toluene, xylenes).

4.2 Results and Discussions

4.2.1 Sludge 1

Results of the primary biodegradation screen for Sludge 1 are summarized in Table 4-2 and illustrated graphically in Figures 4-1, 4-2, and 4-3. Copies of the original laboratory reports are presented in Appendix B.

The 9% load of Sludge 1 exhibited an initial toxicity of 7.5 to 8.0 EC_{50} (Table 4-2). This is in contrast to an EC_{50} value of 2.5 found for the three stock dilutions, 3.0%, 6.25% and 12.5%, of Sludge 1 (Table 3-4). The difference in toxicity is related to the equilibration time of the stock dilutions. Those for the initial characterization were equilibrated overnight in the cold (4°C) prior to analysis. This primary screen sample was equilibrated for 4 days at 4°C. This extended equilibration period was required to assure a stable pH prior to the introduction of nutrients and initiation of biodegradation.

TABLE 4-2

PRIMARY SCREEN FOR THE BIODEGRADATION OF SLUDGE 1

Treatment	Day	Relative Toxicity Gamma Values/Dilution 50	25	12.5	6.25	% EC50	COD (gm/1)	Catalase Activity
Control	0	17.12	4.06	1.83	1.10	7.5	8	5.88
	3	1.69	0.94	0.54	0.26	28.0	2	3.33
	7	1.69	0.98	0.53	0.31	27.0	2	3.56
	10	1.88	0.94	0.49	0.26	25.0	5.9	5.48
	14	0.67	0.33	0.15	0.06	72.0	4.4	3.24
A	0	20.6	4.69	2.11	1.18	8.0	12.5	6.75
	3	1.10	0.51	0.22	0.09	45.0	3	4.00
	7	0.81	0.37	0.21	0.09	70.0	10	4.05
	10	0.43	0.19	0.13	0.08	130.0	8	5.80
	14	0.14	0.046	0.002	0	>100	5.7	3.62
B	0	21.30	4.30	1.87	1.01	7.5	5	5.83
	3	0.90	0.50	0.23	0.13	56.0	2.5	4.05
	7	0.78	0.47	0.27	0.13	80.0	4	3.12
	10	0.37	0.22	0.11	0.8	250.0	5	4.54
	14	0.19	0.06	0.02	0	>100	5.5	4.03
C	0	16.75	3.1	1.49	0.93	7.5	7	5.24
	3	1.19	0.61	0.30	0.17	42.0	2	3.40
	7	0.99	0.57	0.22	0.10	50.0	2	3.62
	10	0.70	0.26	0.11	0.03	66.0	6.5	5.15
	14	0.24	0.02	0.002	0	74.0	4.8	3.95
C_1	0	18.22	3.72	1.59	0.87	7.5	9	5.30
	3	1.25	0.63	0.34	0.17	40.0	2.5	4.99
	7	0.85	0.43	0.31	0.16	60.0	8	4.47
	10	0.39	0.09	0.05	0.02	100.0	8	5.47
	14	0.10	0.04	0.02	0	>100	8.0	3.56
C_2	0	16.64	4.46	1.92	0.92	7.6	5	5
	3	0.90	0.47	0.24	0.07	60.0	2	4.04
	7	0.63	0.46	0.34	0.17	70.0	2	3.56
	10	0.40	0.19	0.11	0.07	150.0	5.3	4.96
	14	0.23	0.11	0.06	0.02	>100	5.7	3.60

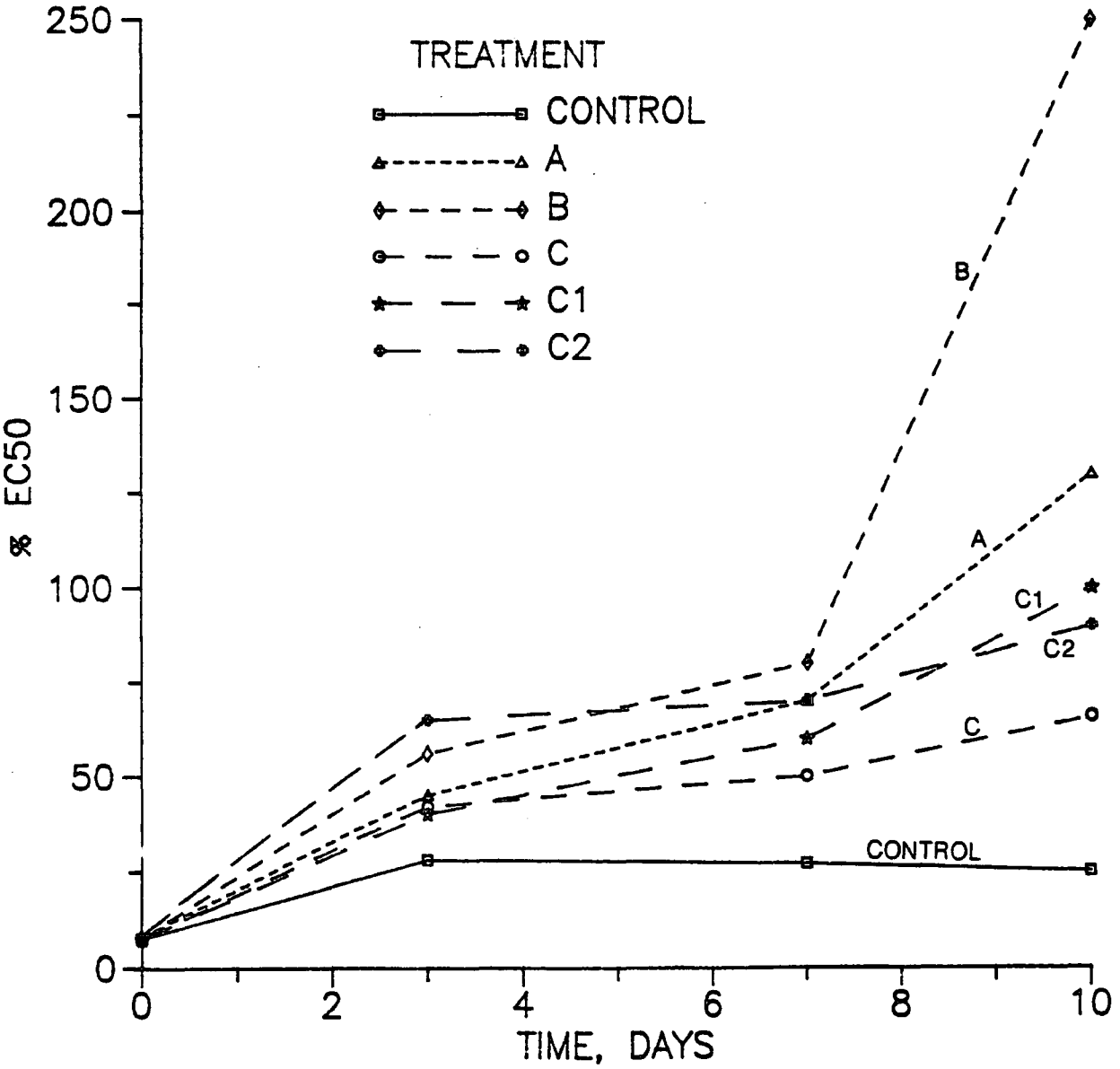

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FIGURE 4-1

RELATIVE TOXICITY OF
LAGOON 1 SLUDGE IN PRIMARY SCREEN
UOP, INC.
DES PLAINES, ILLINOIS

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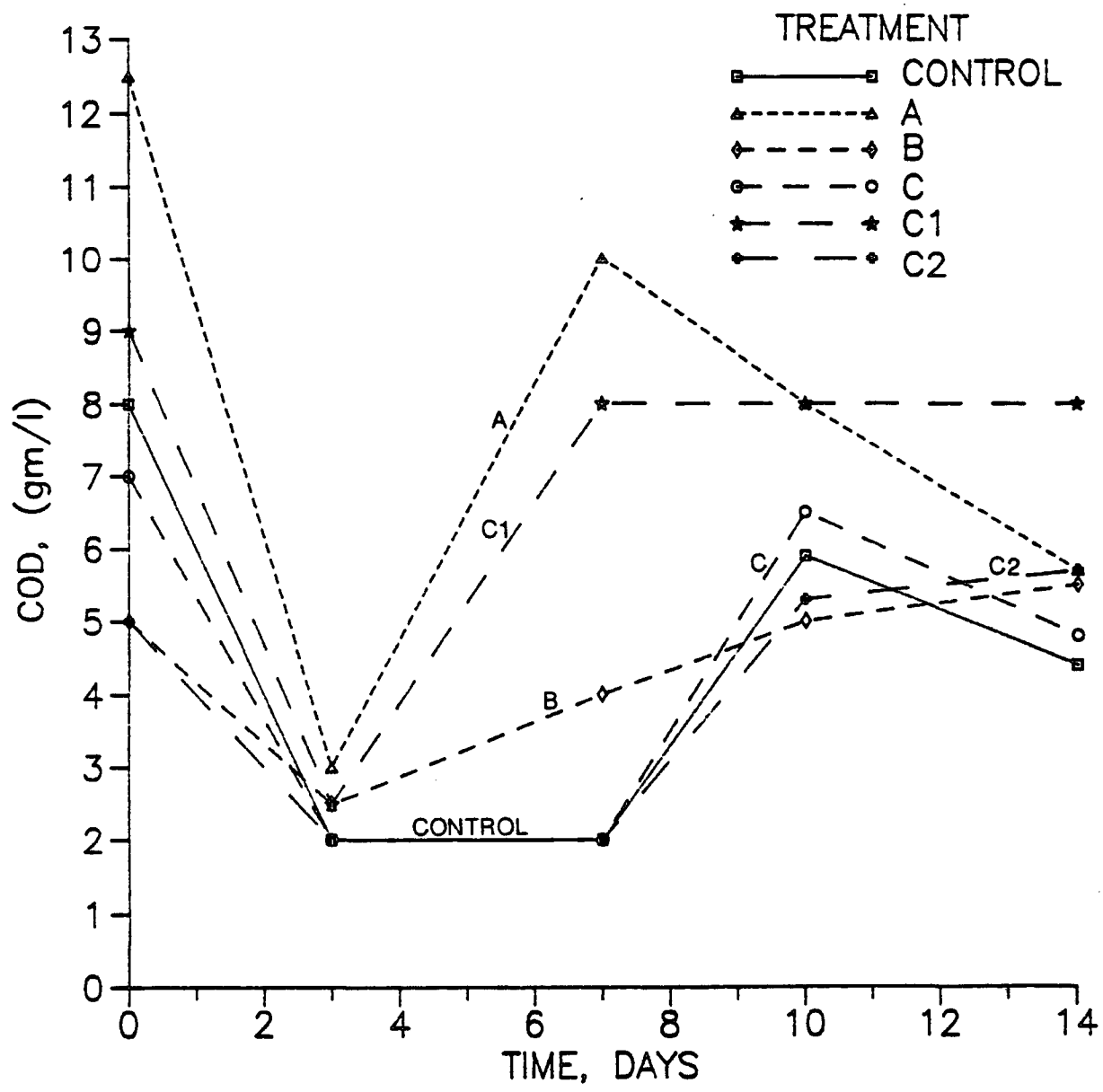

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FIGURE 4-2

CHEMICAL OXYGEN DEMAND (COD) OF LAGOON 1 SLUDGE IN PRIMARY SCREEN

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DES PLAINES, ILLINOIS

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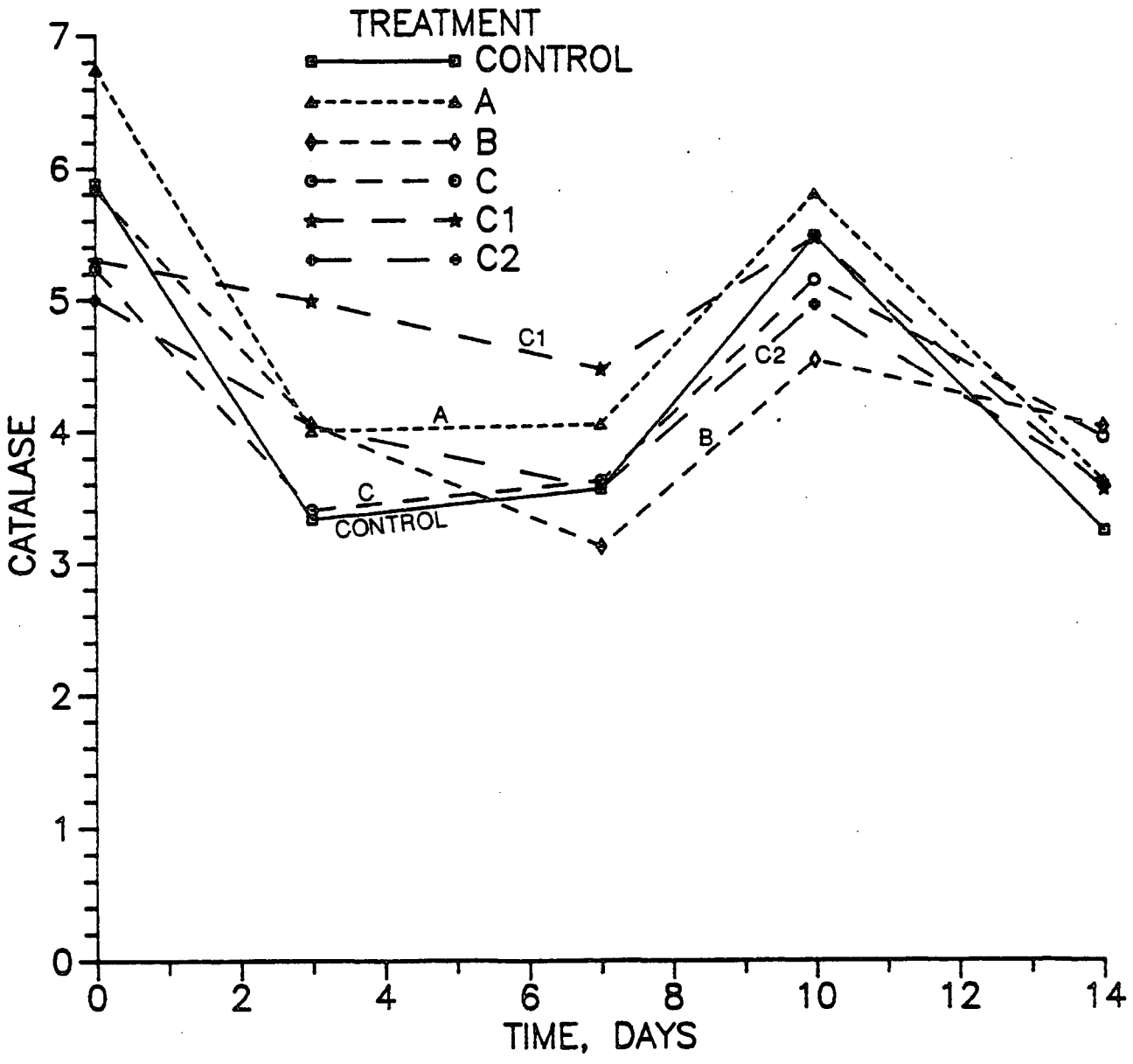

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FIGURE 4-3

CATALASE ACTIVITY OF
LAGOON 1 SLUDGE IN PRIMARY SCREEN
UOP, INC.
DES PLAINES, ILLINOIS

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Four treatments (A, B, C_1, C_2) exhibited a rapid and sustained decrease in toxicity (Figure 4-1). Note that the Effective Concentration 50% (EC_{50}) is an inverse relationship to toxicity of the test material. Highly toxic compounds have a very low Effective Concentration or EC_{50}.

The undisturbed sludge and meadow mat exists in an anaerobic, reducing environment. The chemical oxygen demand (COD) (Figure 4-2) decreased initially as reduced compounds became oxidized. Increasing biological activity and subsequent biological transformation of substrates led to an increase in COD by Day 10. In three treatments (A, C_2 and control), it decreased by Day 14.

Catalase activity of all treatments, except treatment C, decreased initially, then increased and finally decreased by Day 14. Based on these results, the products of treatments A, B and C_2 were analyzed for hydrocarbon oil and grease and BTX (Table 4-4). BTX was below detection limits for all three treatments. Treatment C_2 provided the greatest decrease in hydrocarbon oil and grease.

4.2.2 Sludge 2/Meadow Mat 2

Table 4-3 and Figures 4-4, 4-5, and 4-6 summarize the results of the primary biodegradation screen of the Sludge 2/Meadow Mat 2 mixture. Copies of the original laboratory reports are presented in Appendix B.

All treatments reduced toxicity of the test mixture. But treatment C_2 exhibited the highest, most consistent rate of reduction (Figures 4-4). In other treatments, toxicity decreased initially, stabilized, then decreased again. Since all treatments exhibited a similar trend for chemical oxygen

TABLE 4-3

PRIMARY SCREEN FOR THE BIODEGRADATION OF SLUDGE/
MEADOW MAT COMBINATION FROM LAGOON 2

Treatment	Day	Relative Toxicity Gamma Values/Dilution				% EC50	COD (gm/l)	Catalase Activity
		50	25	12.5	6.25			
Control	0	2.52	0.96	0.40	0.21	30	14	5.74
	3	0.62	0.22	0.04	0	60.0	5	4.07
	7	0.80	0.43	0.26	0.07	52	8	3.27
	10	0.51	0.26	0.09	0.01	80	7.9	4.55
	14	0.44	0.16	0.09	0.05	>100	8.7	4.33
A	0	2.53	0.88	0.36	0.21	30	6	4.40
	3	0.59	0.22	0.11	0	80	5	2.85
	7	0.47	0.17	0.07	0.01	95	6	2.80
	10	0.29	0.07	0.01	0	85	6.2	5.21
	14	0.06	0	0	0	>100	7.7	3.38
B	0	2.60	0.88	0.40	0.18	31	15.5	4.02
	3	0.53	0.23	0.09	0.00	85	3	2.56
	7	0.53	0.19	0.07	0.04	95	6	2.05
	10	0.33	0.14	0.03	0	82	7	4.82
	14	0.12	0.00	0	0	>100	7.1	3.88
C	0	2.72	0.93	0.40	0.20	30	8	4.59
	3	0.52	0.19	0.02	0	65	4	3.82
	7	0.63	0.31	0.13	0.06	70	9	4.33
	10	0.41	0.13	0.07	0.02	90	8.4	4.55
	14	0.27	0.08	0.02	0.02	>100	8.7	4.78
C_1	0	2.59	0.98	0.45	0.23	30	14	4.25
	3	0.54	0.24	0.05	0	75	5	2.84
	7	0.65	0.29	0.12	0.06	77	6	3.65
	10	0.36	0.16	0.06	0.02	95	8.9	4.64
	14	0.16	0.09	0.03	0.02	>100	9.2	3.55
C_2	0	2.43	0.88	0.34	0.22	30	5	5.75
	3	0.60	0.21	0.07	0.04	67	3	3.19
	7	0.49	0.20	0.07	0.01	90	6	2.84
	10	0.32	0.12	0.03	0	110	6.5	4.05
	14	0.14	0.07	0	0	>100	8.2	3.77

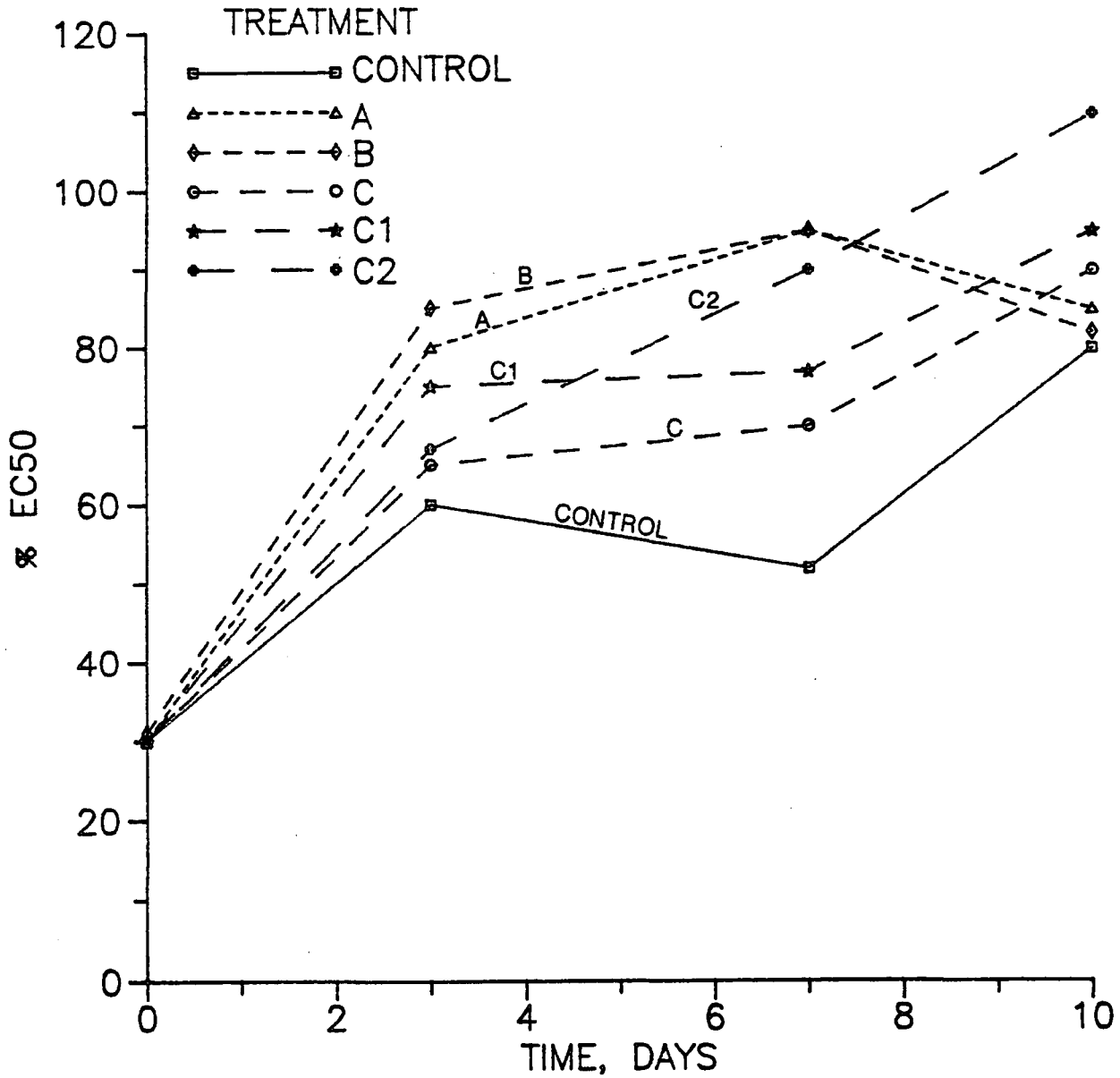

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FIGURE 4-4

RELATIVE TOXICITY OF LAGOON 2 SLUDGE/MEADOW MAT COMBINATION IN PRIMARY SCREEN

UOP, INC.

DES PLAINES, ILLINOIS

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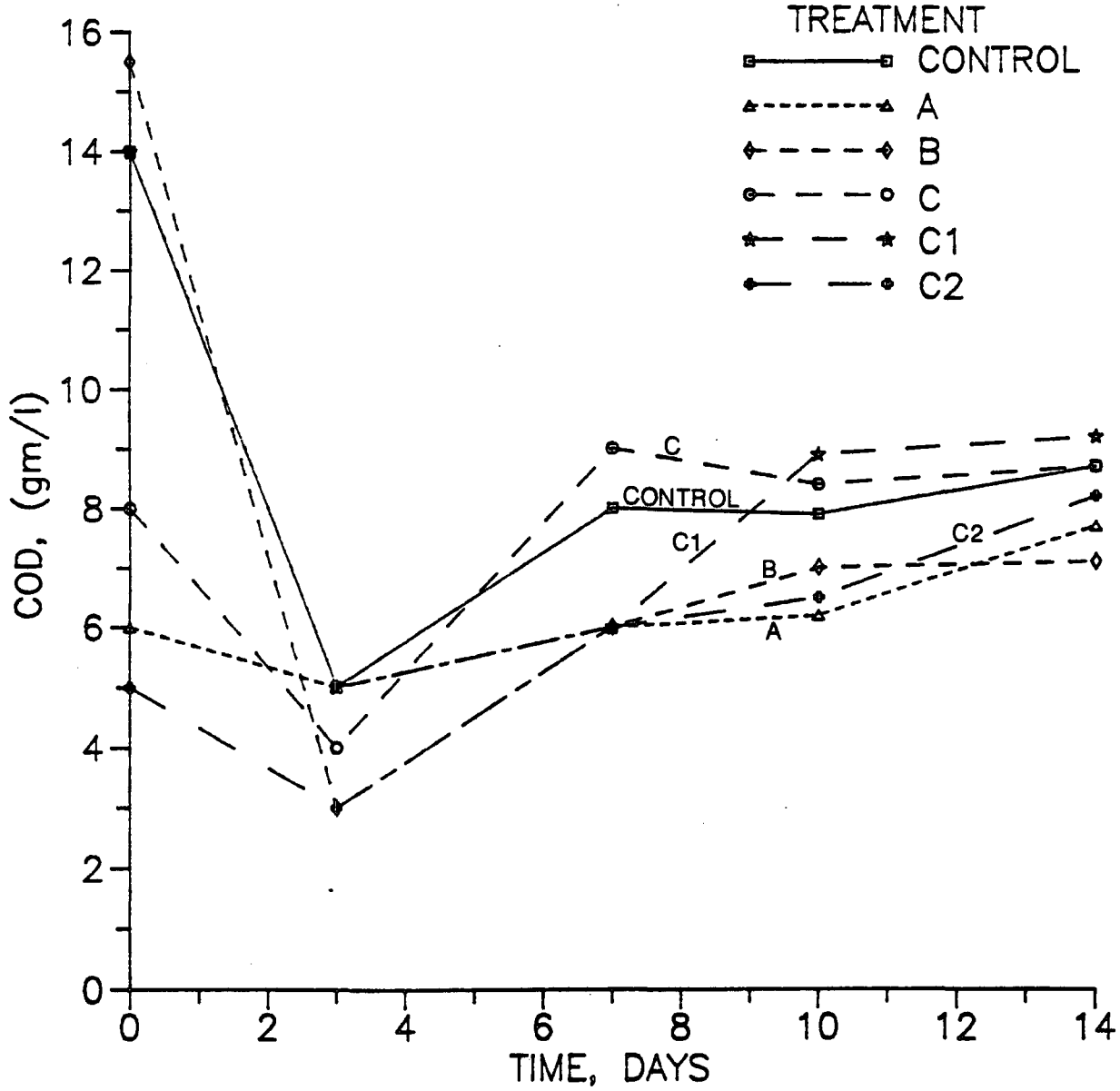

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FIGURE 4-5

CHEMICAL OXYGEN DEMAND (COD) OF LAGOON 2 SLUDGE/MEADOW MAT COMBINATION IN PRIMARY SCREEN

UOP, INC.

DES PLAINES, ILLINOIS

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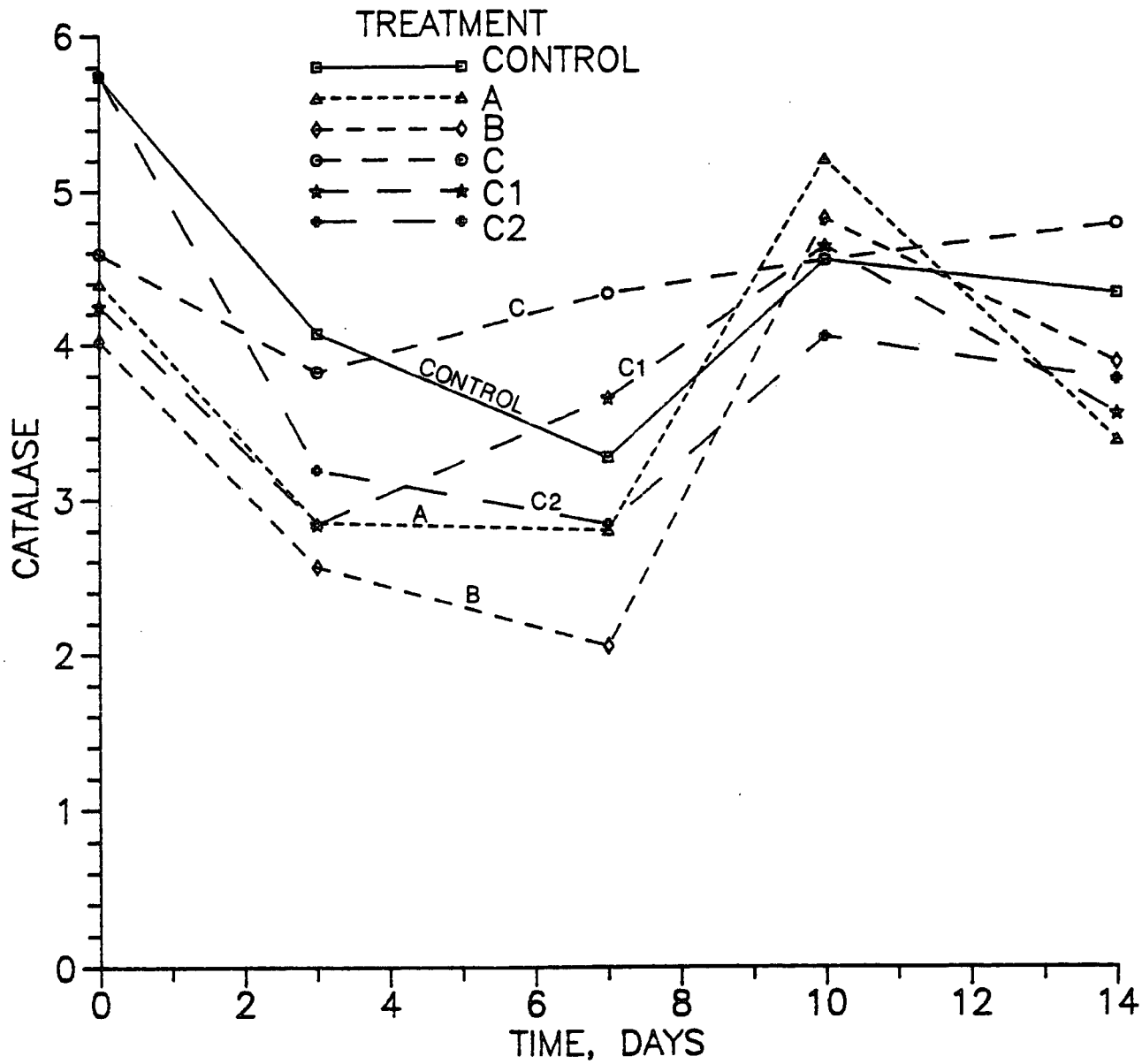

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FIGURE 4-6

CATALASE ACTIVITY OF LAGOON 2 SLUDGE/MEADOW MAT COMBINATION IN PRIMARY SCREEN

UOP, INC.

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demand (COD) and for catalase activity, these assays were ineffective in differentiating treatments. Results of the organic analyses of treatments A, B and C_2 of the Sludge 2/Meadow Mat 2 combination are shown in Table 4-4. Treatment B and C_2 resulted in removal of BTX below detection limits and significant reduction in hydrocarbon oil and grease.

4.2.3 Catalase Results

Results of the catalase assay for both test mixtures were inconsistent with previous observations in similar primary biodegradation screens. The catalase assay generally provides a good indication of biological activity of the various primary screen treatments. Also, one would expect higher catalase activity than the values observed in all treatments of this study after 14 days. The enzyme catalase is found in most cytochrome containing aerobic and facultative anaerobic bacteria, but not in most anaerobic bacteria. The presence of catalase in the microorganisms from the sludge and meadow mat samples was evaluated by placing one drop of 30% hydrogen peroxide on colonies growing on nutrient agar dilution plates. Immediate gas evolution, or bubbling, is a positive indication of catalase activity. Evaluation of microbial colonies from each matrix revealed that less than 7% of the microbial population in Sludge 1 and less than 2% in Sludge 2 were catalase positive. In contrast, nearly 100% of the organisms in the meadow mat were catalase positive. For the catalase assay to be a useful indicator of biological activity, at least 80% of the colonies must be positive. Since sludge is a major component of the contaminated matrix, the use of the catalase assay as a measure of activity is inappropriate.

TABLE 4-4

ORGANIC ANALYSES FOR PRIMARY SCREEN TREATMENTS

Test Mixture	Treatment	Hydrocarb. O&G mg/l		Benzene/Toluene/Xylene (ug/kg)					
				Benzene		Toluene		Xylenes	
		Day 0*	Day 14	Day 0*	Day 14	Day 0*	Day 14	Day 0*	Day 14
Sludge 1	A	479	127	1260	<2	1980	<2	423	<5
	B	479	371	1260	<2	1980	<2	423	<5
	C_2	479	96	1260	<2	1980	<2	423	<5
Sludge 2/MM 2	A	377	61	163	4	90	8	400	15
	B	377	64	163	<2	90	<2	400	<5
	C_2	377	69	163	<2	90	<2	400	<5

*Time zero concentrations were calculated by using the results from the initial sample characterization and then adjusting for the load used in the primary screen: 9% load for sludges (9% sludge, 91% water) and 2% load for meadow mat. Calculations are shown below.

Calculation of Derived Values

	H O&G		Benzene		Toluene		Xylenes	
	Conc.	9%	Conc.	9%	Conc.	9%	Conc.	9%
Sludge 1 Total (ug/kg)	5327	$\frac{479}{479}$	14000	$\frac{1260}{1260}$	22000	$\frac{1980}{1980}$	4700	$\frac{423}{423}$
Sludge 2	3667	330	460	41	440	40	1200	108
	Conc.	2%	Conc.	2%	Conc.	2%	Conc.	2%
Meadow Mat 2 Total (ug/kg)	2344	$\frac{47}{377}$	6100	$\frac{122}{163}$	2500	$\frac{50}{90}$	14600	$\frac{292}{400}$

4.3 Conclusions

Results of the primary biodegradation screen lead to the following conclusions:

1. Treatment C_2 reduced the overall toxicity and provided for significant removal of selected organic contaminants in Sludge 1 alone and in a Sludge 2/Meadow Mat 2 mixture. Since treatment C_2 was the highest concentration tested of a 5:1 nitrogen to phosphorous ratio, higher concentrations may provide additional stimulation. However, excessive nutrient levels can stall the biodegradation process. The biodegradation rates observed are consistent with the near optimum rates observed in other systems. Additional nutrient screen experiments would be required to further refine this nutrient rate.

2. The overall toxicity, as measured by the Microtox bioassay, was significantly reduced by selected nutrient treatments.

3. Catalase activity is an inappropriate measure of microbial activity because most of the indigenous microorganisms in the sludge are catalase negative.

4. Meadow mat does not interfere with removal of organic contaminants and hydrocarbon oil and grease from the sludge. Because of the high microorganism population in meadow mat, it may enhance biodegradation.

5.0 SCALED-UP TEST

Based on the results of the characterization study and the primary biodegradation screen, a scaled-up test was designed to compare promising approaches for field remediation. Samples from Lagoon 1 were chosen for the scaled-up experiment. They had the highest level of toxicity and the highest concentration of organic contaminants.

5.1 Methods

A 1:3.3 mixture consisting of 3,000g of Meadow Mat 1 and 9,900g of Sludge 1 was homogenized in a blender for 10 cycles of 1 min. on, 1 min. off. Nutrients were added as 25.23 ml of the optimum liquid fertilizer from the primary screen (20-9.2-0) to the 12,900g mixture. The pH was 7.0 after mixing. Time zero samples were taken for analysis according to the matrix shown in Table 5-1. The mixture was divided into two treatments: one maintained at 100% field moisture capacity and the other maintained at 50 to 70% of field capacity. The treatments were then placed into opened pans for incubation. Samples were collected from each treatment during the course of the experiment and analyzed according to Table 5-1. Moisture content of the 100% field capacity treatment was maintained by weighing and replacement of evaporative water loss as needed. The dry treatment was permitted to dry naturally to a moisture content of 50 to 70% field capacity as measured with a soil moisture meter. Water was applied as needed. Both treatments were cultivated three times a day to assure mixing and frequent aeration. Both treatments were maintained at pH 7.0 by addition of alkali and incubated at 22 to 24°C for 35 days.

TABLE 5-1

SAMPLING AND ANALYTICAL MATRIX
FOR WET AND DRY TREATMENTS

Day	Microtox	Plate Count	HSL+30	BTX	HO&G	Nutrients
0*	x	x	x		x	x
3	x					
7	x					
10	x					
14	x	x			x	x
21	x			x	x	
29	x					
35	x	x	x		x	

* Only one sample per analysis was taken from the initial mixture (day 0) because the dry treatment developed as the process progressed.

5.2 Results and Discussions

Table 5-2 provides the calculations used to determine the day zero calculated values in Tables 5-3 and 5-5. Analytical data from the individual characterization of Sludge 1 and Meadow Mat 1 was used.

Table 5-3 summarizes Microtox, HO&G and BTX results for the 35-day treatment period. Toxicity remained constant in the wet matrix. However, it decreased significantly after Day 10 in the dry treatment as shown in Figure 5-1. Concentration of hydrocarbon oil and grease decreased steadily from the calculated value in both treatments. BTX fell below detectable limits after 35 days in both treatments (Figure 5-2). Toluene and xylene, however, were present in both treatments at Day 21.

Table 5-4 shows the nutrient and microbial plate count data for both treatments. The wet treatment had a higher concentration of soluble ammonia, nitrate, and phosphorus than the dry. This could be attributed to a lower rate of nutrient utilization and incorporation into biomass in the wet treatment.

Table 5-5 summarizes results of the HSL analyses at Day 0 and Day 35. Volatiles and BNA extractables decreased in both treatments as shown in Figures 5-3 and 5-4. Pesticides reported, were attributed to matrix interferences. The absence of pesticides was confirmed by further analysis by mass spectrum. (See Appendix C.)

Compounds tentatively identified by their elution characteristics on gas chromatography/mass spectroscopy (GC/MS) are summarized in Tables 5-6, 5-7, and 5-8 for the Day 0 and Day 35 wet and dry treatments, respectively. The biodegradation treatment reduced the total concentration of compounds detected by GC/MS. The dry treatment reduced the concentration 10 fold. The wet treatment reduced the concentration 30 fold.

TABLE 5-2

CALCULATED VALUES BASED ON
INITIAL SAMPLE CHARACTERIZATION

Analysis	Meadow Mat #1		Sludge #1		Day 0 Calculated Concentration[3]
	CONC[1]	Adj. Amt[2]	CONC[1]	Adj. Amt[2]	
Hyd. O & G (mg/kg)	1,825	425	5,327	4,086	4,511
Benzene (ug/kg)	8,750	2,039	14,000	10,738	12,777
Toluene (ug/kg)	84,000	19,572	22,000	16,874	36,446
Xylenes (ug/kg)	5,700	1,328	4,700	3,605	4,933
NH_3-N (mg/kg)	369	86	29	22	108
NO_3-N (mg/kg)	246	57	103	79	136
P (mg/kg)	110	26	106	81	107
CFU/gm	$14x10^5$	$3x10^5$	$19.0x10^7$	$14.6x10^7$	$14.6x10^7$

1. Values from characterization data, Tables 3-3 and 3-4.
2. Characterization concentration times percent of final mixture.
3. Sum of columns 2 and 4.
4. Calculations:

3,000g Meadow Mat #1 $\frac{3,000}{12,900} = 23.3\%$ Meadow Mat #1
9,900g Sludge #1 $\frac{9,900}{12,900} = 76.7\%$ Sludge #1
12,900g Total

TABLE 5-3

SCALED-UP SOLID MATRIX TREATMENT
OF LAGOON 1 SLUDGE/MEADOW MAT MIXTURE

					BENZENE/TOLUENE/XYLENE					
	MICROTOX EC_{50}[1]		HO&G		BENZENE		TOLUENE		XYLENES	
			mg/kg		ug/kg		ug/kg		ug/kg	
DAY	Dry[2]	Wet[2]	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
CALC.[3]			NA	4,511	NA	12,777	NA	36,446	NA	4,933
0	18.7	18.7	NA	1,960	NA	3,800	NA	10,000	NA	3,400
3	15.7	24.2	NA	NA	NA	NA	NA	NA	NA	NA
7	18.8	27.8	NA	NA	NA	NA	NA	NA	NA	NA
10	22.7	24.2	NA	NA	NA	NA	NA	NA	NA	NA
14	72.2	26.1	3,130	3,370	NA	NA	NA	NA	NA	NA
21	80.8	24.6	2,800	2,300	1	7	20	45	18	77
29	80.3	22.5	NA	NA	NA	NA	NA	NA	NA	NA
35	81.7	26.5	1,550	1,680	5	50	5	50	5	50

[1]Adjusted to 10g dry weight sample

[2]Dry (50-70% field moisture capacity) = Wet (100% field capacity)

[3]From initial matrix characterization using 23.3% Meadow Mat #1, 76.7% sludge #1 (See Table 5-2)

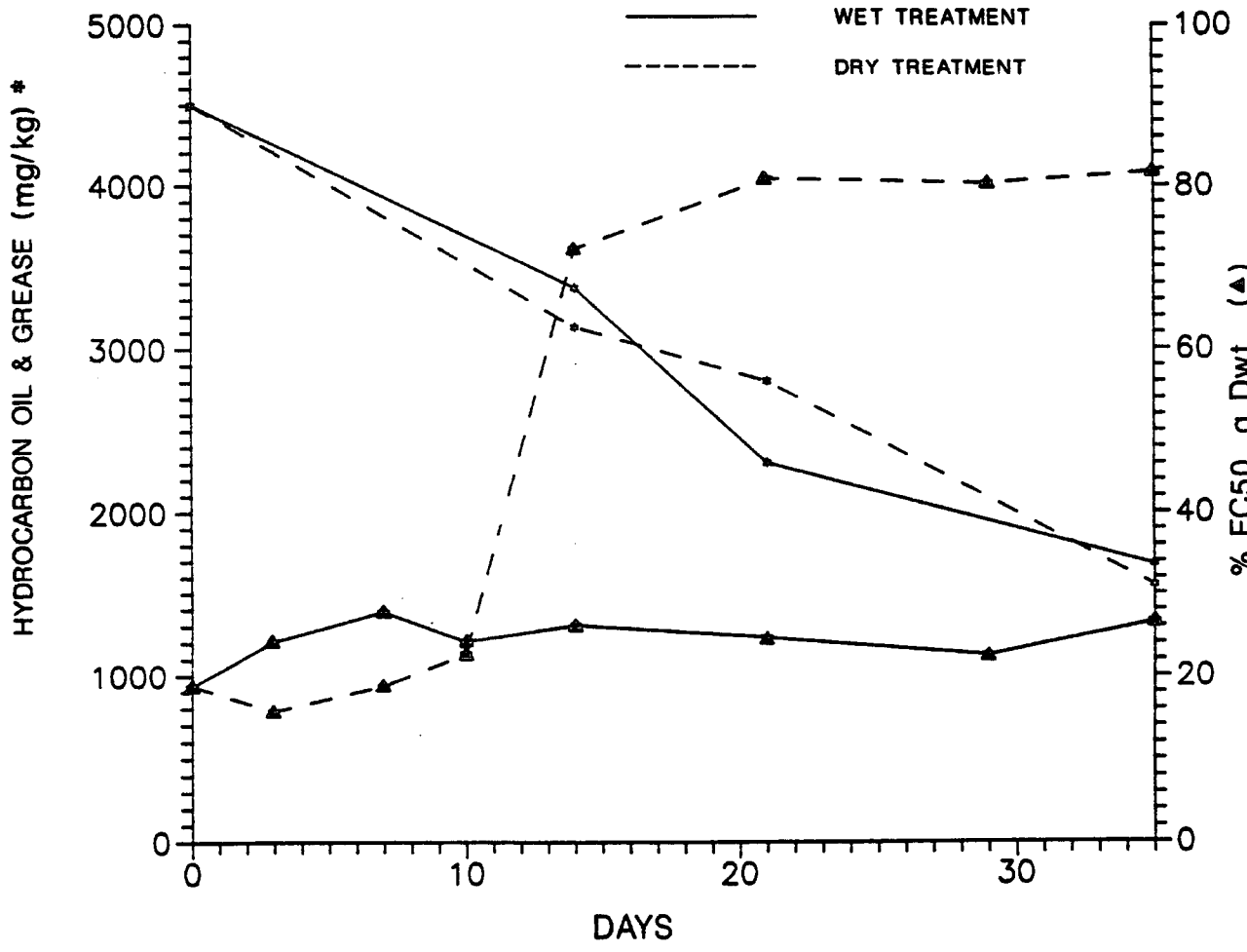

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FIGURE 5-1
TOXICITY AND HYD. OIL & GREASE
SCALED UP TEST
UOP, INC.
DES PLAINES, ILLINOIS

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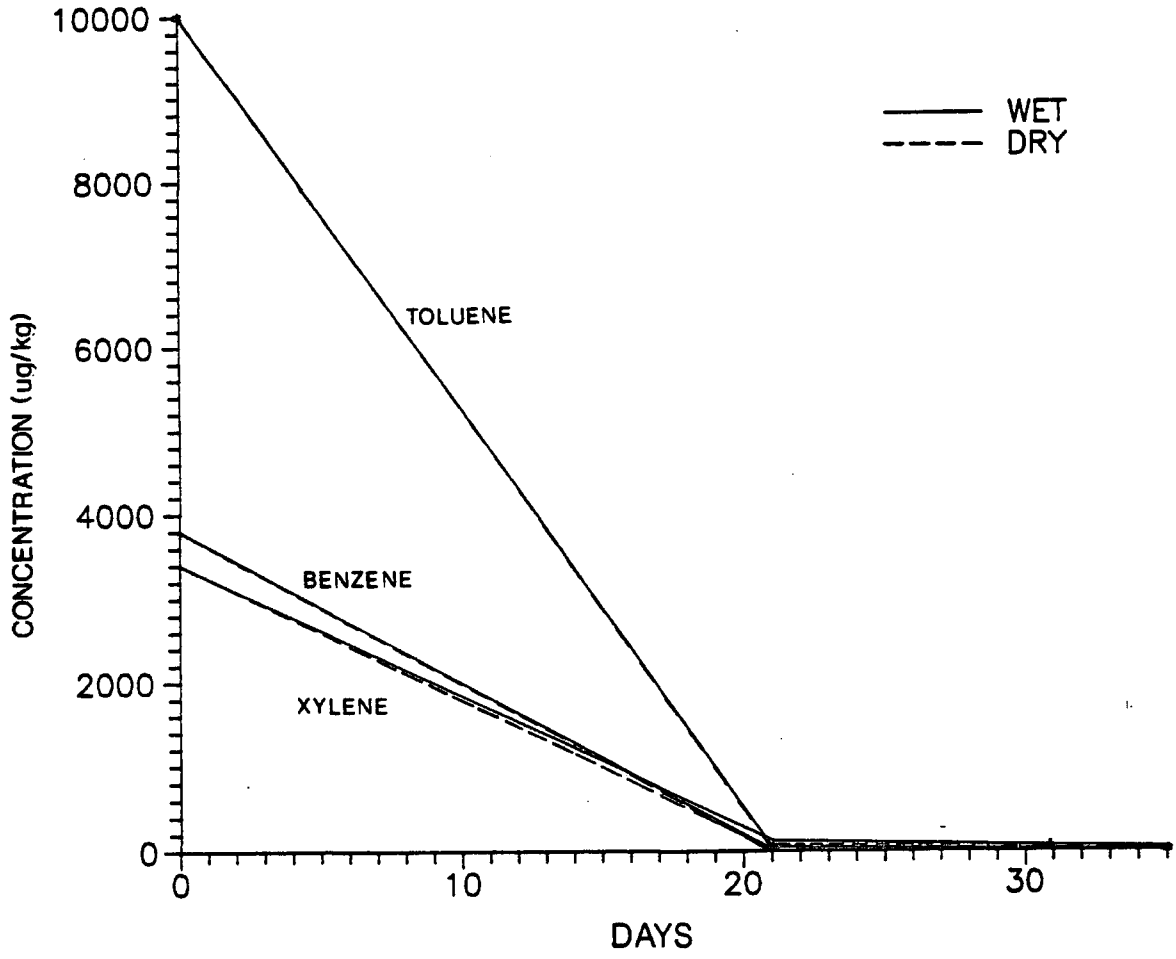

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FIGURE 5-2

BENZENE, TOLUENE, XYLENE
SCALED UP TEST

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TABLE 5-4

NUTRIENTS AND CELL COUNTS FOR
SCALED UP TREATMENT

Day	NH_3-N (ppm)		NO_3-N (ppm)		P (ppm)		CFU x 10^8/gm	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet
Calc.	NA*	108	NA*	136	NA*	107	NA*	5.93
0	NA*	730	NA*	3,070	NA*	11,300	NA*	2.60
14	525	1,450	655	1,037	2,718	7,118	1.72	2.77
35	NA	NA	NA	NA	NA	NA	1.80	2.09

* No samples existed for the dry treatment at time zero. This is because the dry treatment developed as moisture was lost during incubation and cultivation.

TABLE 5-5

HSL+30 SUMMARY

COMPOUND* (ug/kg)	TIME AND TREATMENT 0	35W	35D
Benzene	3,800	<50	<5
Chlorobenzene	2,400	<50	<5
Ethylbenzene	2,000	<50	<5
Toluene	10,000	<50	<5
Methylene Chloride	1,000	970	34
Total Volatiles**	18,200	970	34
o-Xylene	3,400	<50	<5
4-Methylphenol	<33,000	<540	470
Bis (2-ethylhexyl)phthalate	<33,000	<540	400
1,2-dichlorobenzene	43,000	7,000	<330
2,4-dinitrotoluene	<33,000	1,200	500
Total Base/Neutral/Acid Extractables**	46,400	8,200	1,370
Total Tentatively Identified Compounds**	1,778,000	65,000	179,000
Percent on HSL***	3.5%	12.4%	0.8%

* All other compounds on the HSL were < detection limit for all times and treatments.

** Below detection limit concentrations were taken as zero for totals.

*** Percentage of HSL compounds in the total organics detected by the HSL+30 methodology.

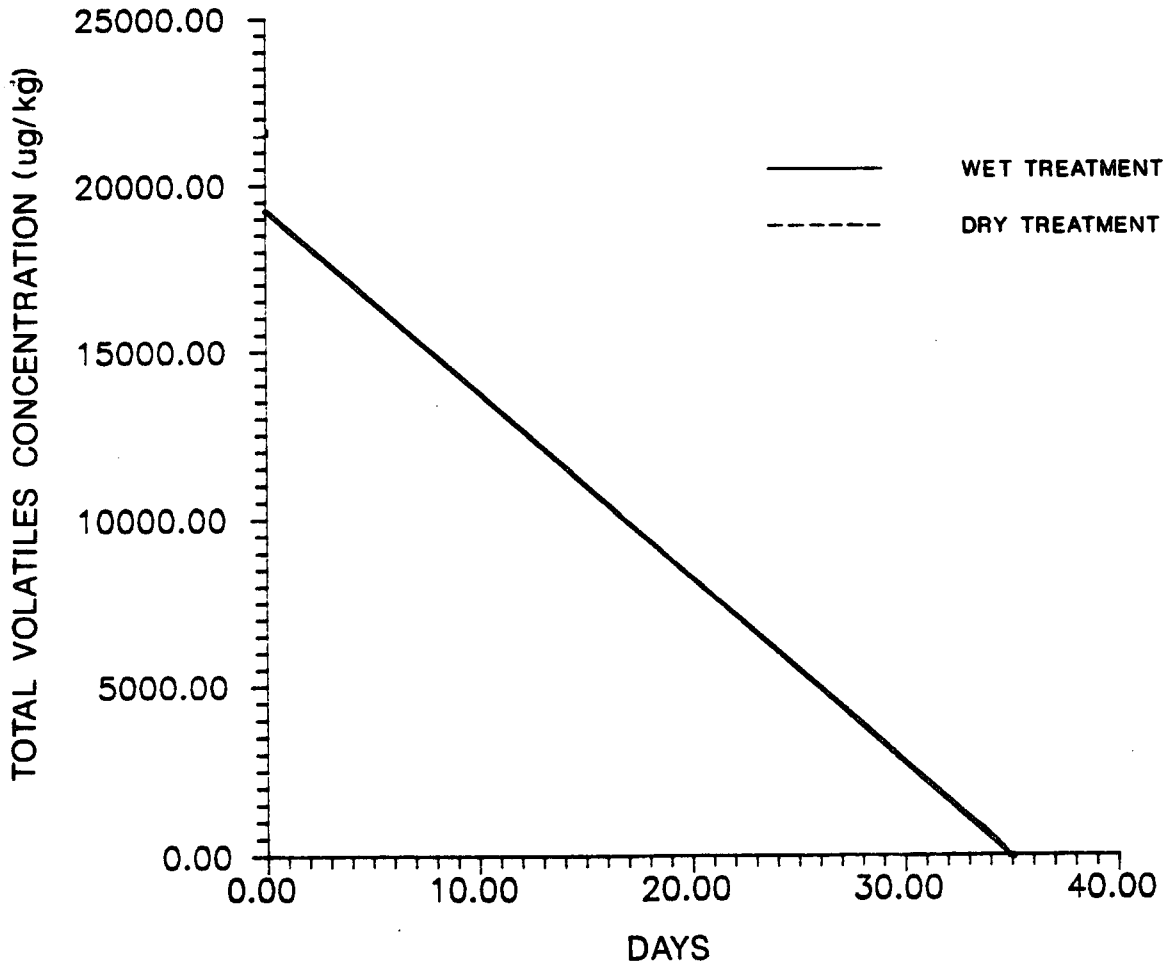

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FIGURE 5-3

TOTAL VOLATILES
SCALED UP TEST

UOP, INC.
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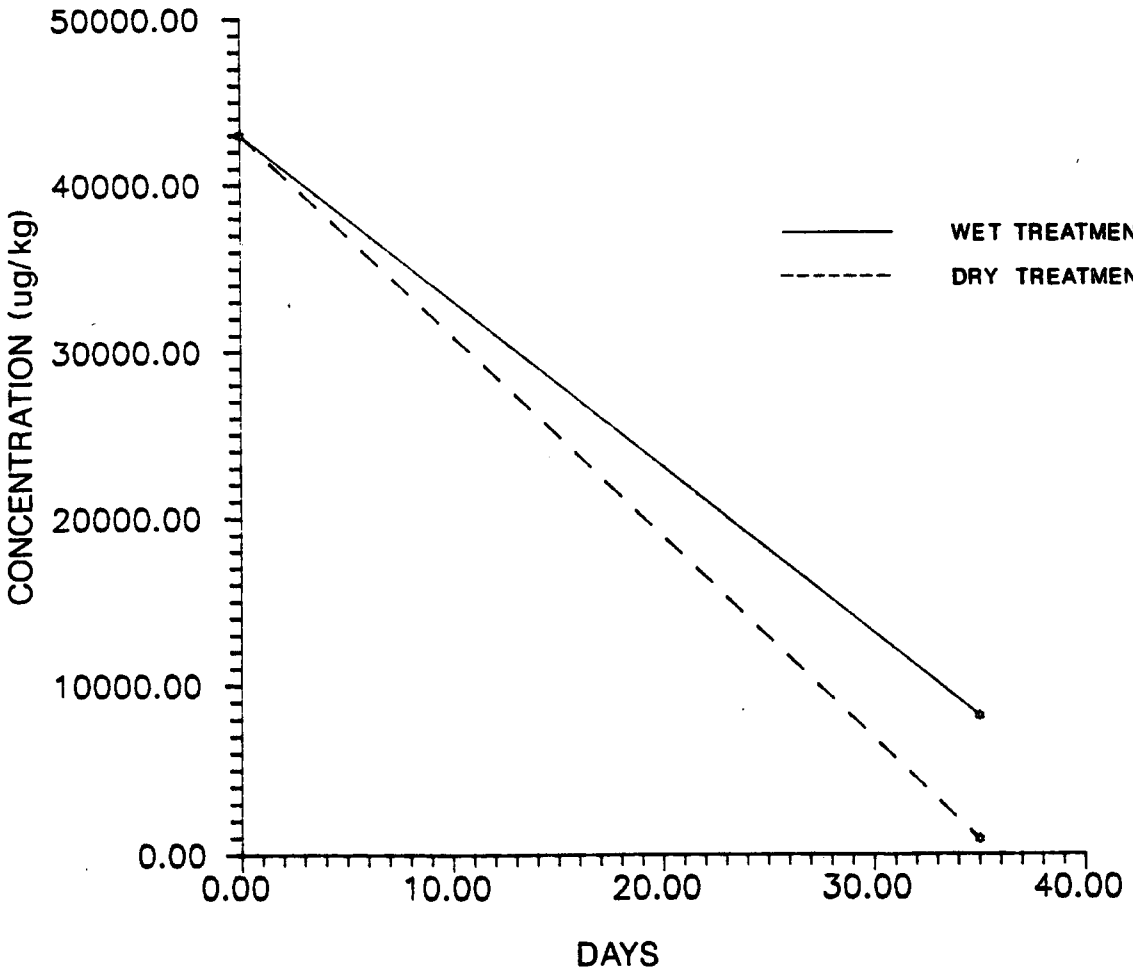

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FIGURE 5-4

BASE, NEUTRAL AND ACID EXTRACTABLES
SCALED UP TEST

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TABLE 5-6
SUMMARY OF TENTATIVELY IDENTIFIED COMPOUNDS
LISTED BY CONCENTRATION IN DESCENDING ORDER
SCALED-UP TEST, DAY 0

Compound*	Estimated Concentration, mg/kg	Purity**	Fraction***
Benzene, 1-(1,1-dimethylethyl)-3-methyl-[isomer]	748	828	2
Not identified	260	-	2
2-Pentanone, 4-Hydroxy-4-methyl-	190	956	2
Benzene, 1,1-[methylenebis (oxymethylene)]Bis-	140	884	2
Not identified	115	-	2
Benzene, 1,4-Dibutoxy-	110	906	2
Benzene, 1-(1,1-dimethyl ethyl)-3-methyl-[isomer]	80	901	2
Benzene, 1,2-dichloro-	50	944	2
Benzophenone	40	929	2
Benzene, 1-chloro-2-methyl-	9.4	884	1
Not identified	7.5	-	2
Not identified	5.1	-	1
Benzene, 1,2-dimethyl-	4.9	805	2
Benzene	4.4	815	2
Not identified	4.3	-	2
Methylene Chloride	3.6	-	2
Not identified	3.5	-	2
Not identified	2.5	-	2
18 Compounds	1,778		

*In some instances, more than one compound could be identified on one chromatograph; only the most likely identified compound is listed.
**Purity indicates the likelihood of a correct identification. A perfect match provides a value of 1000, therefore, the higher the purity value, the greater the likelihood of correct identification. Purity values greater than 800 are usually considered to be good matches.
***1. = Volatile Compound, 2. = Semi-volatile Compound.

TABLE 5-7

SUMMARY OF TENTATIVELY IDENTIFIED COMPOUNDS
LISTED BY CONCENTRATION IN DESCENDING ORDER
SCALED-UP TEST, DAY 35, WET MATRIX

Compound*	Estimated Concentration, mg/kg	Purity**	Fraction***
Benzene, 1-(1,1-dimethylethyl)-3-methyl-	30	883	2
Benzene, (Butoxymethyl)-	4.2	820	2
Benzophenone	4.2	818	2
Benzene, 1,2-dichloro-	4.0	889	2
Not identified	2.9	-	2
Not identified	2.9	-	2
Not identified	2.4	749	2
Benzene, 1,1-methylenebis-	2.2	884	2
Benzene, 1-(1-ethylpropyl)-4-methyl-	1.7	845	2
Not identified	1.7	-	2

*In some instances, more than one compound could be identified on one chromatograph; only the most likely identified compound is listed.
**Purity indicates the likelihood of a correct identification. A perfect match provides a value of 1000, therefore, the higher the purity value, the greater the likelihood of correct identification. Purity values greater than 800 are usually considered to be good matches.
***1. = Volatile Compound, 2. = Semi-volatile Compound.

TABLE 5-7 (Continued)

Compound*	Estimated Concentration, mg/kg	Purity**	Fraction***
Methylene Chloride	1.5	864	1
Heptane, 4-(1-methyl ethyl)-	1.3	925	2
Not identified	1.0	-	2
Not identified	0.91	-	2
Benzene, 1,4-Dibutoxy-	0.90	848	2
Phosphoric Acid	0.70	837	2
Not identified	0.63	-	2
Not identified	0.60	-	2
Not identified	0.4	-	1
Not identified	0.39	-	2
20 Compounds	65		

TABLE 5-8

SUMMARY OF TENTATIVELY IDENTIFIED COMPOUNDS

LISTED BY CONCENTRATION IN DESCENDING ORDER

SCALE-UP TEST, DAY 35, DRY MATRIX

Compound*	Estimated Concentration, mg/kg	Purity**	Fraction***
Not identified	67.0	-	2
Not identified	38.0	-	2
Not identified	13.7	-	2
Not identified	12.2	-	2
Not identified	8.0	-	2
Not identified	4.4	-	2
Not identified	4.1	-	2
Not identified	3.3	-	2
Not identified	3.2	-	2
Not identified	2.8	-	2
Not identified	2.3	-	2
Not identified	2.3	-	2
Not identified	2.2	-	2
Not identified	2.0	-	2
Not identified	2.0	-	2
Not identified	1.9	-	2

*In some instances, more than one compound could be identified on one chromatograph; only the most likely identified compound is listed.

**Purity indicates the likelihood of a correct identification. A perfect match provides a value of 1000, therefore, the higher the purity value, the greater the likelihood of correct identification. Purity values greater than 800 are usually considered to be good matches.

***1. = Volatile Compound, 2. = Semi-volatile Compound.

TABLE 5-8 (Continued)

Compound*	Estimated Concentration, mg/kg	Purity**	Fraction***
Not identified	1.8	-	2
Not identified	1.5		2
Not identified	1.4	-	2
Not identified	1.3	-	2
Not identified	1.2	-	2
Not identified	0.90	-	2
Not identified	0.80	-	2
Methylene Chloride	0.51	863	1
Acetone	0.10	873	1
25 Compounds	179		

Of the total organics detected by GC/MS (HSL+30) at day 0, 3.5% were accounted for on the HSL. After 35 days of dry treatment, 0.8% of the total organics (HSL+30) remained on the HSL. In contrast, 12% of the total organics detected, remained on the HSL after 35 days of wet treatment. The dry treatment preferentially stimulated biodegradation of the listed organics compared to the wet treatment.

The total concentration of organic compounds detected (HSL+30) in the dry treatment was 2.4 times greater than in the wet treatment. This may be due to continued biodegradation and biological transformation of higher molecular weight organics from the sludge and meadow mat.

Only a small window of organic compounds is evaluated by this method. Higher molecular weight compounds exhibit elution times greater than those examined by the column used in this analysis. Biodegradation and biological transformation of these higher molecular weight compounds produced smaller molecular weight intermediates that may be detected in this assay now. This could be confirmed by extending the elution time or by using an alternative column to separate larger compounds. Consequently, selection of the optimum treatment (wet or dry) based solely on the decrease in total concentration of organic compounds detected by GC/MS, compared to the initial concentration, is inappropriate. The most important indicator is the decrease in HSL compounds.

5.3 Conclusions

1. The initial toxicity characterization predicted that organic components of sludges and meadow mats were biodegradable without dilution to reduce overall toxicity. A scaled-up biodegradation demonstration of the most toxic sludge/meadow mat mixture confirmed this.

2. In the scaled-up test, 85 ppm hydrocarbon oil and grease was consumed per day. At this rate, reduction of 4,600 ppm to a residual of 1,000 ppm would require 42 days, 100 ppm 53 days.

3. Volatile organic compounds as represented by benzene, toluene, and xylene were almost completely removed during the first 21 days of incubation.

4. Overall toxicity of the Lagoon 1 Sludge/Meadow Mat mixture decreased rapidly after 14 days of incubation at 50 to 70% field moisture capacity (dry treatment).

5. Data from the tentatively identified compounds support the trend shown by those on the HSL. Biological treatment in either the wet or dry matrix reduced the total concentration of organic compounds as analyzed by GC/MS. The dry treatment decreased the percentage of HSL compounds in the total organics (HSL+30) more than the wet treatment.

6. The HSL+30 analysis showed that: a) there is no accumulation of recalcitrant or undegradable compounds; b) biodegradation does not simply "delist" the organic contaminants by transformation; and c) there is no accumulation of organic, biodegradation intermediates or products.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Meadow mat does not interfere with the biological process by diverting microbial metabolic activity. The additional biomass provided by the meadow mat may enhance biological activity.

   Rationale: Because of its high, readily available carbon content, the meadow mat was considered to be a preferred substrate of the indigenous organisms. Stimulation of the microorganisms by the addition of nutrients and oxygen could lead to the preferential utilization of meadow mat at the exclusion of the organic contaminants. This was not the case. The sludge/meadow mat combination, from Lagoon 2 in the primary screen and Lagoon 1 in the scaled-up test exhibited favorable biodegradation of the organic contaminants.

   The meadow mat has a higher population of microorganisms than the sludge, 10^7 versus 10^5 colony-forming units per gram. The fact that most of the meadow mat organisms are catalase positive suggests the presence of oxidative metabolic capabilities essential to the rapid biodegradation of organic contaminants. The readily degradable carbon compounds in the meadow mat may act as the important cometabolites required for the biodegradation of the organic contaminants.

2. Addition of nutrients and oxygen accelerated the removal of organic contaminants and reduced the toxicity in both the primary biodegradation screen and the scaled-up confirmation.

   Rationale: While direct measurement of biodegradation activity (catalase assay) and microbial growth (plate count) is inconclusive, there is significant evidence supporting biodegradation. Maintenance of a high steady state population of 10^8 colony forming units/g during the 35 day scaled-up test suggests minimal endogenous activity. Utilization of available carbons and other nutrients is required to sustain a biomass of this magnitude. Transformation activity, as shown by the final HSL+30 analysis of the wet and dry treatments of the scaled-up test, indicate that oxidative metabolism is a significant removal mechanism for organic constituents.

3. Of the two treatments in the scaled-up test the dry treatment matrix provided the most rapid removal of organic contaminants.

   Rationale: More rapid removal of organics from the dry treatment coupled with a higher microbial population supports oxidation by aerobic metabolism as a significant pathway to remediation. Organic compounds were removed from the wet treatment matrix at a lower rate. The data suggest, however, that acceptable decontamination levels can be reached by either treatment, given sufficient incubation time.

4. Volatilization is one mechanism of removal of the organic constituents, however, it is unlikely to be the major removal route for the following reasons:

   a. The low vapor pressure of many of the compounds found in the HSL+30 analysis suggests low volatilization at ambient temperatures.

   b. Adsorption of the organic compounds to the sludge/meadow mat matrix inhibits volatilization.

   c. The high water content of both treatments in the scaled-up test favors solubilization and reduces sublimation.

   d. The initial concentration of specific organic contaminants is low enough that the partitioning effects of adsorption and solubilization became significant compared to volatilization.

5. This study shows that bioremediation is an effective course of action for removing contaminants from the lagoon's sludge and meadow mat.

6.2 Recommendations For Remediation

Careful management of the sludge and meadow mat layers of the wastewater lagoons will accelerate the natural bioremediation of the organic contaminants. The following recommendations address specific parameters that will stimulate bioremediation.

1. Mixing. To initiate bioremediation, the sludge and meadow mat fractions should be thoroughly mixed and then cultivated at least 3 times daily.

2. Nutrient Amendments. The following rates of nitrogen and phosphorus will stimulate biological activities in the sludge/meadow mat mixture:

1,242 lbs nitrogen/acre ft
571 lbs phosphate (P_2O_5)/acre ft

This 5:1 ratio of nitrogen to phosphorus should be formulated as a liquid fertilizer and sprayed as a thin film over the surface of the treatment area. Daily cultivation practices will provide for adequate incorporation of the nutrients into the treatment mixture.

3. pH Control. Monitor pH during the biodegradation process to assure neutral to slightly alkaline conditions. Biodegradation processes frequently lead to acidic conditions, be prepared to adjust the pH with hydrated calcitic lime. Apply the lime as a slurry as uniformly as possible over the surface of the treatment area. Regular cultivation will provide sufficient mixing for incorporation.

4. Moisture Conditions. Maintain the moisture content of the treatment matrix at 50-70% of field capacity. Excessive moisture limits the transfer of oxygen and carbon dioxide. Aerobic conditions favor the most rapid metabolic activity and provide for the most complete biodegradation. Anaerobic conditions, like

those created with excessive moisture, will extend the treatment period.

5. Treatment System Operations. Monitor the progress of biodegradation by measuring toxicity and hydrocarbon oil and grease. When the EC_{50} is greater than 80% and the hydrocarbon oil and grease is less than 1,500 mg/kg conditions are appropriate for analysis of specific organic constituents to confirm attainment of decontamination objectives.

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